

HELICOBACTER PYLORI

Relationship between *Helicobacter pylori* babA2 status with gastric epithelial cell turnover and premalignant gastric lesions

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Background: *Helicobacter pylori* blood group antigen binding adhesin (BabA) mediates bacterial adherence to human blood group antigens on gastric epithelium. Although strains harbouring *babA2* were recently found to be associated with peptic ulcer and gastric cancer, the role of *babA2* in cellular turnover, severity of gastritis, and premalignant changes is poorly understood.

Aim: We correlated *H pylori* *babA2*, vacuolating toxin (*vacA*), and cytotoxin associated gene A (*cagA*) genotypes with the severity of gastric inflammation and epithelial cell turnover in a group of Chinese patients from an area with a high incidence of gastric cancer.

Patients and methods: *H pylori* isolates were obtained from 104 Chinese patients who participated in a gastric cancer prevention programme. Genotype variants of *babA2*, *vacA*, and *cagA* were determined by polymerase chain reaction. Antrum and corpus histopathology was examined according to the updated Sydney classification. Apoptosis was scored by terminal uridine deoxynucleotidyl nick end labeling (TUNEL) and proliferation by Ki-67 immunostaining.

Results: Of the 104 patients, 102 (98.1%) harboured *cagA*⁺ strains and all had *vacA* s1 genotype. The *babA2*⁺ strains were found in 83 (79.8%) patients and were associated with higher lymphocytic infiltration ($p=0.028$), presence of glandular atrophy (odds ratio (OR) 7.5, 95% confidence interval (CI) 2.3–24.3), and intestinal metaplasia (OR 7.4, 95% CI 2.2–25.3) in the antrum. Increased epithelial proliferation was also noted in individuals infected with *babA2*⁺ strains ($p=0.025$). Strains harbouring *cagA*⁺/*vacA* s1 genotypes lacked this association in the absence of *babA2*.

Conclusions: The presence of *babA2*⁺ *H pylori* strains alone or in combination with *cagA*⁺ and *vacA* s1 was associated with the presence of preneoplastic gastric lesions.

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Helicobacter pylori colonises the human stomach and is the principal cause of chronic gastritis, peptic ulcer disease, gastric atrophy (AT), intestinal metaplasia (IM), and gastric adenocarcinoma. However, *H pylori* infection is usually asymptomatic and only a minority of patients develops serious clinical outcomes. The reasons for this may be related to differences in genetic susceptibility of the host,¹ environmental factors,^{2–4} and genetic diversity of *H pylori*.^{5–6} In this context, the relevance of specific *H pylori* virulence associated genes has been extensively studied. The cytotoxin associated gene A (*cagA*) was the first to be identified and was shown to be associated with duodenal ulcers and gastric carcinoma.^{7–8} The vacuolating toxin (*VacA*) was subsequently discovered. Allelic variants of the *vacA* gene were identified in the signal region (s1, s2) and mid region (m1, m2) of the gene which appear to regulate cytotoxic activity.⁹ Specifically, *vacA* s1/m1 strains have higher cytotoxic activity than s1/m2 strains whereas s2/m2 strains have no cytotoxic activity.⁹

Apart from bacterial toxins, recent studies have provided evidence that bacterial adherence factors may also contribute to the pathogenicity of *H pylori*. The blood group antigen binding adhesin (BabA) has been shown to mediate adherence of *H pylori* to human blood group antigens on gastric epithelial cells.^{10–11} Two corresponding genes encoding BabA have been cloned: *babA1* and *babA2*, but only the *babA2* gene is functionally active.¹² Recently, *babA2* was found to be significantly associated with duodenal ulcer and gastric cancer.¹³ None the less, the importance of *H pylori* adherence factors to gastric epithelial cell turnover, degree of gastric inflammation, glandular AT, and IM is poorly understood. It is further complicated by the observations that the frequency of these virulence determinants and their association with gastrointestinal pathologies appears to vary considerably between different geographic regions. In

the present study, we correlated *babA2*, *cagA*, and *vacA* genotypes with gastric inflammation, presence of glandular atrophy and IM, and gastric epithelial cell turnover in Chinese patients.

MATERIALS AND METHODS

Study design and subjects

This was a cross sectional study that examined the correlation between various *H pylori* genotypes, gastric inflammation, and cellular turnover. Subjects were previously recruited in a randomised control trial of *H pylori* eradication in the prevention of gastric cancer in Yantai County, Shandong province of China, where the incidence of gastric cancer is among the highest in China.¹⁴ Endoscopy was performed in all subjects, with gastric biopsies obtained. Two antral and two corpus biopsies were fixed in 10% buffered formalin and processed for histological examination. One extra antral biopsy was used for bacterial culture for *H pylori*.

In this study, 104 *H pylori* positive subjects (median age 50 years; range 39–75) were studied. These patients were randomly selected from those with persistent active *H pylori* infection at first follow up, as detected by bacterial culture.¹⁵ None of these patients had previously received anti-*Helicobacter* therapy.

H pylori culture

One antral biopsy was placed in selective transport medium and cultured on horse blood agar at 37°C under micro-

Abbreviations: IM, intestinal metaplasia; *cagA*, cytotoxin associated gene A; *VacA*, vacuolating toxin; AT, atrophy; TdT, terminal deoxynucleotidyl transferase; TUNEL, triphosphate nick end labeling; AI, apoptosis index; PI, proliferation index; OR, odds ratio; BabA, blood group antigen binding adhesin; PCR, polymerase chain reaction.

Table 1 Primer sequences for polymerase chain reaction based genotyping of *Helicobacter pylori* isolates

Ref	Gene	Sequence (5' to 3')	Product size
13	<i>vacA</i> s1/s2	Forward ATGGAATACAACAACACAC	259 bp (s1)
		Reverse CTGCTTGAATGCGCCAAAC	286 bp (s2)
17	<i>vacA</i> m1/m2	Forward CAATCTGTCCAATCAAGCGAG	570 bp (m1)
		Reverse GCGTCTAAATAATTCCAAGG	645 bp (m2)
13	<i>cagA</i>	Forward ATAATGCTAAATTAGACAACCTGAGCGA	297 bp
		Reverse TTAGAATAATCAACAACATCACGCCAT	
16	<i>babA2</i>	Forward AATCCAAAAAGGAGAAAAAGTATGAAA	850 bp
		Reverse TGTTAGTGATTTCGGTGTAGGACA	

aerophilic conditions, as previously described.¹⁵ *H pylori* was identified by typical colony morphology, Gram stain, and positive biochemical testing for urease, catalase, and oxidase. Bacteria were harvested from the plates using sterile cotton swabs and stored at -70°C in brucella broth plus 30% (vol/vol) fetal calf serum and 20% (vol/vol) glycerol. All frozen isolates were controlled for contamination.

Detection of *H pylori* genotypes and sequencing

H pylori *cagA*, *vacA*, and *babA2* genotypes were determined by polymerase chain reaction (PCR). Genomic DNA was extracted from *H pylori* using the High Pure PCR Template Preparation Kit (Roche, Germany). The integrity of the DNA was assessed by 1.2% agarose gels stained with ethidium bromide. PCR reactions were performed in a total volume of 50 μl which contained 50 pmol of primers, 100 ng of genomic DNA, 1.0 mM of each of the four dNTPs, and 2 units of AmpliTaq DNA polymerase (Perkin-Elmer, Norwalk, Connecticut, USA). Primer sequences were described previously^{13 16 17} and are listed in table 1 for reference. The amplified PCR products were resolved in 1.5% agarose gels, stained with ethidium bromide, and visualised under a short wavelength ultraviolet light source. The sequences of the PCR products were confirmed by automated sequencing (ABI Prism 310 Genetic Analyzer; Perkin Elmer, Branchburg, New Jersey, USA) using the same primer pairs.

Histological assessment

Haematoxylin-eosin stained sections of the gastric antrum and corpus biopsies were examined by a single pathologist (KFT) who was blinded to the other experimental results. Slides were coded and evaluated histologically for the following parameters: (1) activity of gastritis (granulocytic cell infiltration); (2) chronicity of gastritis (lymphocytic and plasma cells infiltration); (3) glandular AT; and (4) IM. Each parameter was graded

as none (0), mild (1), moderate (2), or severe (3) according to the updated Sydney classification.¹⁸ Glandular atrophy was defined as loss of gastric glands with replacement by metaplastic epithelium or fibrosis. IM was defined as the presence of foci where at least three neighbouring gastric pits contained two or more goblet cells in each pit.

Assessment of apoptosis and determination of apoptosis index

Apoptosis was determined by the terminal deoxynucleotidyl transferase (TdT) mediated deoxyuridine triphosphate nick end labeling (TUNEL) technique (ApopTag; Intergen, New York, USA), as described previously.¹⁹ In addition, positively stained cells should also bear morphological characteristics of apoptosis, including chromatin condensation, nuclear disintegration, and formation of crescentic caps of condensed chromatin at the nuclear periphery. The apoptosis index (AI) was calculated as the ratio of TUNEL positive nuclei over the total number of cells counted (usually more than 1000).

Assessment of proliferation and determination of proliferation index

Proliferation was assayed by immunoperoxidase staining for Ki-67, a proliferation associated antigen that has been used extensively in the assessment of proliferation in the stomach.^{19 20} In brief, paraffin embedded sections were dewaxed, dehydrated, and then subjected to microwave antigen retrieval. Endogenous peroxidase was blocked by 3% hydrogen peroxide. Normal rabbit serum (5%) was applied to prevent non-specific binding of primary monoclonal antibodies. The tissue was incubated with monoclonal mouse anti-human Ki-67 antibody (Clone: mib 1, isotype: IgG1, without dilution; Zymed, San Francisco, California, USA). Biotinylated rabbit antimouse antibody (dilution 1:200; Dako, Glostrup, Denmark) was applied as secondary antibody and followed by application of avidin-biotinylated peroxidase complex (dilution 1:100; Dako). The slides were developed in 3,3'-diaminobenzidine tetrahydrochloride (DAB, Dako) and counterstained with Mayer haematoxylin. The proliferation index (PI) was expressed as a percentage of the ratio of Ki-67 positive nuclei to the total nuclei counted.¹⁹

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Science program (SPSS, version 10.0) and MINITAB (Release 7.2, Minitab, Inc). As gastric histology, particularly AT and IM, may be confounded by the effect of age, we have adjusted for the effect of age in this study. Mantel-Haenszel statistics were used to assess the relationship between individual genotypes and the presence of activity, chronicity, glandular AT, and IM after adjusting for the effect of age; the results are expressed as common odds ratios (OR) with the corresponding 95% confidence intervals (CI).

Table 2 Distribution of different *Helicobacter pylori* genotypes in 104 patients

Genotype	No (%)
<i>babA2</i> ⁺	83 (79.8)
<i>cagA</i> ⁺	102 (98.1)
<i>vacA</i>	
s1	104 (100)
s2	0 (0)
m1*	43 (41.3)
m2*	58 (55.8)
<i>cagA</i> ⁺ / <i>vacA</i> s1	102 (98)
<i>cagA</i> ⁺ / <i>vacA</i> m1	43 (41.3)
<i>babA2</i> ⁺ / <i>cagA</i> ⁺ / <i>vacA</i> s1	82 (78.8)
<i>babA2</i> ⁺ / <i>cagA</i> ⁺ / <i>vacA</i> s1	20 (19.2)

*There were three strains that could not be typed.

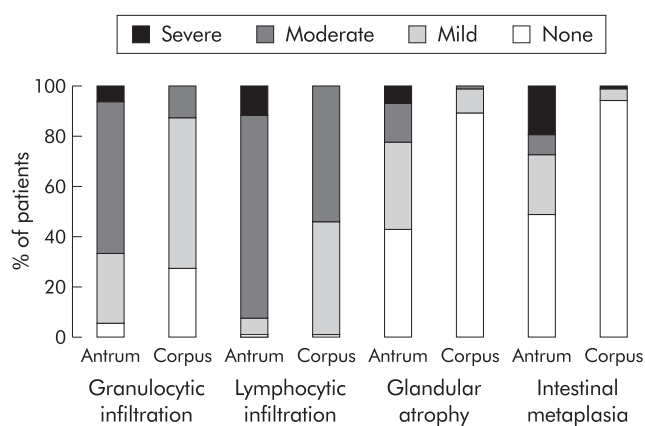


Figure 1 Distribution of the severity of gastritis in all study patients (n=104). The degree of granulocytic infiltration (activity), lymphocytic infiltration (chronicity), glandular atrophy, and intestinal metaplasia was graded as none (0), mild (1), moderate (2), or severe (3), as suggested by the updated Sydney classification.¹⁸

The age of the subjects was stratified into two groups (>50 or ≤50 years old) according to the median age of the study population. The Mann-Whitney U test was used to compare the severity of gastritis among different genotypes. Spearman's correlation coefficient was used to assess the correlation between (1) proliferation and severity of inflammation; and (2) age and AT/IM scores. The Pearson correlation coefficient was used to examine the correlation between epithelial cell proliferation and apoptosis. The two tailed independent *t* test was performed to examine the difference in AI and PI among various genotypes. A two sided *p* value of less than 0.05 was considered statistically significant.

RESULTS

Characteristics of *H. pylori* isolates

Of the 104 *H. pylori* infected subjects, 102 (98.1%) harboured *cagA*⁺ strains. All *H. pylori* isolates belonged to the *vacA* s1 genotype and thus 98.1% were *cagA*⁺/*vacA* s1. For the middle (m) region of the *vacA* gene, 101 isolates could be typed and the remaining three strains could not. There were 43 (42.6%) m1 and 58 (57.4%) m2 strains. Eighty three (79.8%) were *babA2*⁺ strains and the simultaneous presence of *cagA*, *vacA* s1, and *babA2* genes (triple positive) was found in 82 (78.8%) *H. pylori* isolates (table 2). The presence of the *babA2* genotype did not correlate with the presence of *cagA* or various *vacA* genotypes.

Histological assessment of gastritis

Most *H. pylori* infected patients had evidence of granulocytic and lymphocytic infiltration in the stomach (fig 1). Granulocytic infiltration was seen in 94.2% of antral biopsies and 72.1% of corpus biopsies. All except one *H. pylori* positive patient had lymphocytic infiltration. In general, the severity of inflammation was more marked in the antrum than in the body. Fifty three patients had IM and/or AT in the antrum

whereas 12 patients had IM and/or AT in the body. The severity of glandular atrophy in the corpus, but not in the antrum, correlated with the age of the patient (antrum *r*=0.09, *p*=0.35; corpus *r*=0.24, *p*=0.014). On the other hand, there was no significant correlation between IM and patient age (antrum *r*=0.09, *p*=0.39; corpus *r*=0.18, *p*=0.08).

Correlation between *H. pylori* genotypes and inflammation

As most *H. pylori* infected patients had evidence of acute and chronic inflammatory infiltrates in the stomach, there was no significant correlation between different *H. pylori* genotypes and the presence of granulocytic and lymphocytic infiltrates in the antrum or body of the stomach (*p*>0.3). However, the presence of the *babA2*⁺ genotype was associated with a higher degree of lymphocytic infiltration (chronicity) in the antrum (*p*=0.028, table 3).

Correlation between *H. pylori* genotypes and atrophy/metaplasia

After adjustment for age, carriage of *babA2*⁺ *H. pylori* isolates was associated with a higher frequency of glandular AT (OR 7.5, 95% CI 2.3–24.3) and IM (OR 7.4, 95% CI 2.2–25.3) in the antrum (table 4). A similar association was also demonstrated in triple positive (*babA2*⁺/*cagA*⁺/*vacA* s1) *H. pylori* isolates. The corresponding OR values for AT and IM in triple positive strains were 7.1 (95% CI 2.2–23.3) and 7.0 (95% CI 2.1–24.1). None of the *H. pylori* genotype or genotype combinations appeared to correlate with preneoplastic lesions in the corpus.

Epithelial cell proliferation and apoptosis

The association between cellular proliferation and apoptosis was examined. There was a significant correlation between PI and AI (*r*=0.37, *p*=0.001) but there was no direct correlation between proliferation and severity of inflammation (both activity and chronicity).

babA2 genotypes and cell proliferation

Proliferation indexes of different genotypes are shown in fig 2. PI was significantly higher in *babA2*⁺ isolates (mean (SD) 21.3 (17.7)) than in *babA2*[−] isolates (12.2 (13.9); mean difference 9.1, 95% CI 1.46–16.70; *p*=0.025). In addition, there was a significant difference in PI between the *babA2*⁺/*cagA*⁺/*vacA* s1⁺ (21.4 (17.9)) and *babA2*[−]/*cagA*⁺/*vacA* s1⁺ (12.9 (13.9)) isolates (mean difference 8.5, 95% CI 0.64–16.29; *p*=0.041). There was however no difference in proliferation between *vacA* m1 (21.6 (18.6)) and *vacA* m2 (16.5 (15.6)) strains (mean difference 5.1, 95% CI −2.73–12.95; *p*=0.19).

babA2 genotypes and apoptosis

Cellular apoptotic indexes of different *H. pylori* genotypes are demonstrated in fig 3. In contrast with proliferation, there was no difference in AI between *babA2*⁺ and *babA2*[−] isolates (mean (SD) 0.67 (0.61) v 0.66 (0.52); mean difference 0.01, 95% CI −0.27–0.29; *p*=0.96). In addition, AI was comparable between *babA2*⁺/*cagA*⁺/*vacA* s1⁺ and *babA2*[−]/*cagA*⁺/*vacA* s1⁺ (0.68 (0.61) v 0.67 (0.53); mean difference 0.00, 95% CI −0.28–0.29; *p*=0.98),

Table 3 *Helicobacter pylori* genotypes and median (range) gastritis score

	<i>babA2</i> ⁺ (n=83)	<i>babA2</i> [−] (n=21)	<i>p</i> Value	<i>vacA</i> m1 (n=43)	<i>vacA</i> m2 (n=58)	<i>p</i> Value	<i>babA2</i> ⁺ / <i>cagA</i> ⁺ / <i>vacA</i> s1 (n=82)	<i>babA2</i> [−] / <i>cagA</i> ⁺ / <i>vacA</i> s1 (n=20)	<i>p</i> Value
Antrum									
Activity	2.00 (0–3)	2.00 (0–3)	0.49	2.00 (0–3)	2.00 (0–3)	0.12	2.00 (0–3)	2.00 (0–3)	0.59
Chronicity	2.00 (1–3)	2.00 (0–3)	0.028	2.00 (1–3)	2.00 (0–3)	0.34	2.00 (1–3)	2.00 (0–3)	0.065
Corpus									
Activity	1.00 (0–2)	1.00 (0–2)	0.73	1.00 (0–2)	1.00 (0–2)	0.77	1.00 (0–2)	1.00 (0–2)	0.77
Chronicity	2.00 (1–2)	2.00 (0–2)	0.82	1.00 (1–2)	2.00 (0–2)	0.59	2.00 (1–2)	2.00 (0–2)	0.99

Comparison between different groups was made using the Mann-Whitney U test.

Activity=degree of granulocytic cell infiltration; chronicity=degree of lymphocytic and plasma cell infiltration.

Table 4 Odds ratio of glandular atrophy (AT) and intestinal metaplasia (IM) in different *Helicobacter pylori* genotype combinations after adjustment for age

	AT (%)	Non-AT (%)	Odds ratio* (95% CI)	IM (%)	Non-IM (%)	Odds ratio* (95% CI)
Antrum						
<i>babA2</i> ⁺	54 (65.1)	29 (34.9)		49 (59.0)	34 (41.0)	
<i>babA2</i> ⁻	5 (23.8)	16 (76.2)	7.5 (2.3–24.3)	4 (19.0)	17 (81.0)	7.4 (2.2–25.3)
<i>vacA</i> m1	25 (58.1)	18 (41.9)		22 (51.2)	21 (48.8)	
<i>vacA</i> m2	32 (55.2)	26 (44.8)	1.1 (0.5–2.4)	30 (51.7)	28 (48.3)	0.9 (0.4–2.1)
<i>babA2</i> ⁺ / <i>cagA</i> ⁺ / <i>vacA</i> s1	54 (65.9)	28 (34.1)		49 (59.8)	33 (40.2)	
<i>babA2</i> ⁻ / <i>cagA</i> ⁺ / <i>vacA</i> s1	5 (25.0)	15 (75.0)	7.1 (2.2–23.3)	4 (20.0)	16 (80.0)	7.0 (2.1–24.1)
Corpus						
<i>babA2</i> ⁺	8 (9.6)	75 (90.4)		5 (6.0)	78 (94.0)	
<i>babA2</i> ⁻	3 (14.3)	18 (85.7)	0.8 (0.2–3.3)	1 (4.8)	20 (95.2)	1.5 (0.2–14.2)
<i>vacA</i> m1	5 (11.6)	38 (88.4)		2 (4.7)	41 (95.3)	
<i>vacA</i> m2	6 (10.3)	52 (89.7)	1.0 (0.3–3.6)	4 (6.9)	54 (93.1)	0.6 (0.1–3.3)
<i>babA2</i> ⁺ / <i>cagA</i> ⁺ / <i>vacA</i> s1	8 (9.8)	74 (90.2)		5 (6.1)	77 (93.9)	
<i>babA2</i> ⁻ / <i>cagA</i> ⁺ / <i>vacA</i> s1	3 (15.0)	17 (85.0)	0.7 (0.2–3.1)	1 (5.0)	19 (95.0)	1.4 (0.2–13.2)

*Common odds ratio was given after adjustment for the effect of age (>50 or ≤50 years) by the Mantel-Haenszel statistics.

95% CI, 95% confidence interval.

As all strains were of the *vacA* s1 genotype and 98.1% strains were *cagA*⁺, analysis was not possible for these two genotypes.

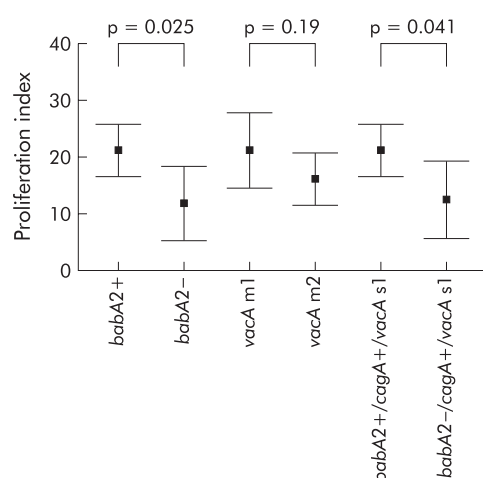


Figure 2 Proliferation index in different *Helicobacter pylori* genotypes. Results are plotted as mean and 95% confidence interval. The squares indicate the mean and the horizontal lines the upper and lower values of the 95% confidence interval. Significant differences were noted in the proliferation index between (a) *babA2*⁺ and *babA2*⁻ ($p=0.025$); and (b) *babA2*⁺/*cagA*⁺/*vacA* s1 and *babA2*⁻/*cagA*⁺/*vacA* s1 ($p=0.041$).

and between *vacA* m1 and *vacA* m2 (0.62 (0.54) v 0.65 (0.49); mean difference -0.02, 95% CI -0.26–0.21; $p=0.86$) isolates.

DISCUSSION

In the present study, we have examined three genotypes (*cagA*, *vacA* and *babA2*) that have been suggested to have higher pathogenic potential in *H pylori* isolates from northern China. All *H pylori* isolates were of the *vacA* s1 genotype and 98% were *cagA*⁺, indicating that the *cagA*⁺/*vacA* s1 genotypes are highly prevalent in this population with a high background incidence of gastric cancer. Additionally, we found that approximately 80% of patients were infected with *babA2*⁺ strains and 41.3% patients were harbouring *vacA* m1 strains. Carriage of the *babA2*⁺ strains was associated with more intense chronic inflammation (table 3), and presence of glandular AT and IM in the gastric antrum (table 4). This association with preneoplastic gastric lesions was also demonstrated in *H pylori* isolates that were triple positive (*babA2*⁺/*cagA*⁺/*vacA* s1) compared with *cagA*⁺/*vacA* s1 strains that lacked *babA2*. Recently, a similar association between gastric inflammation and *babA2* genotype was reported in a German study.²¹ The investigators reported that type I (*cagA*⁺/*vacA* s1) *H pylori* strains that were negative for *babA2* lacked the association with gastric inflammation and preneoplastic lesions. In contrast

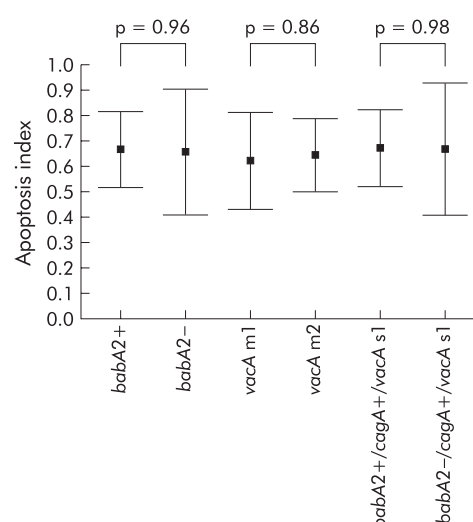


Figure 3 Apoptosis index in different *Helicobacter pylori* genotypes. Results are plotted as mean and 95% confidence interval. There was no significant difference in apoptosis index between (a) *babA2*⁺ and *babA2*⁻; (b) *babA2*⁺/*cagA*⁺/*vacA* s1 and *babA2*⁻/*cagA*⁺/*vacA* s1; and (c) *vacA* m1 and *vacA* m2.

with the German study that reported a 38% prevalence of the *babA2* gene, *babA2* was present in approximately 80% of our study population. In a recent Japanese study, the prevalence of the *babA2* genotype was reported to be 85%.²² The differential prevalence of *babA2*⁺ *H pylori* isolates in these three different studies appears to parallel the gastric cancer incidence of the countries. Countries with a high background incidence of gastric cancer such as Japan and China tend to have a higher frequency of *babA2*⁺ *H pylori*. As attachment to gastric epithelial cells by *H pylori* is mediated by BabA, it has been postulated that this attachment process precedes all other putative pathogenic processes.²¹ Intuitively, adhesion to epithelial cells via BabA may facilitate the effective delivery of bacterial products such as the CagA protein into the host cells and the subsequent tyrosine phosphorylation process.²³ None the less, *babA2* is probably only one of the mediators for the attachment process and other unidentified factors are also likely to be involved. Otherwise, one might expect to find a wider difference in inflammation scores between infection by *babA2*⁺ and *babA2*⁻ isolates (table 3).

Age is one of the factors that may be associated with the presence of AT and IM in *H pylori* infected individuals. In our previous study, we showed that older subjects are more likely to demonstrate progression of metaplasia than younger

subjects.¹⁴ However, even after adjustment for age, the frequencies of AT and IM were still significantly higher in individuals harbouring the *babA2*⁺ strains or the triple positive *H. pylori* strain. In this study, we stratified patients into two groups, with 50 years as the dividing line, because this was the median age of our subjects. Moreover, in a previous decision analysis modelling study of *H. pylori* screening to prevent gastric cancer, the investigators suggested that screening at age 40–50 years is considered to be reasonably cost effective.²⁴ Therefore, it appears that most gastric changes related to chronic *H. pylori* infection had already developed at 50 years.

Disturbance in the balance between cellular proliferation and apoptosis may predispose to either cell loss with mucosal damage or cell accumulation and cancer development.^{25–26} Recent studies suggested that *H. pylori* infection increases gastric mucosal apoptosis in vivo.^{19–27–30} However, the effect of *H. pylori* on gastric epithelial cell proliferation is conflicting.^{19–27–30–33} One explanation for this discrepancy may be related to the presence of different bacterial genotypes.^{34–35} However, whether *H. pylori* adherence factors, such as BabA, affect cellular kinetics is unclear. Our findings demonstrated that subjects harbouring *H. pylori babA2*⁺ strains had significantly higher gastric proliferation rates than subjects with *babA2*[−] strains, which was unrelated to the degree of inflammation. More importantly, this increase in gastric cell proliferation was not accompanied by a parallel increase in apoptosis. Although the triple positive strains (*babA2*⁺/*cagA*⁺/*vacA*s1) had a similar increase in cell proliferation, the conventional type I (*cagA*⁺/*vacA*s1) *H. pylori* strains which were negative for *babA2* lacked such an association. In other words, in subjects infected with *H. pylori babA2*⁺ strains, there was dissociation between gastric epithelial cell proliferation and apoptosis, which may predispose to cancer development.

In conclusion, *babA2*⁺ *H. pylori* isolates were present in approximately 80% of the study population from Yantai County where the incidence of gastric cancer is among the highest in China. The presence of *babA2*⁺ *H. pylori* strains alone, or in combination with *cagA*⁺/*vacA*s1, was associated with the presence of glandular AT and IM, and heightened gastric epithelial proliferation in the antrum. Carriage of *babA2*⁺ *H. pylori* strains and the associated changes in the gastric epithelium may partly explain the high background incidence of gastric cancer in the study population.

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REFERENCES

- Azuma T, Ito S, Sato F, et al. The role of the HLA-DQA1 gene in resistance to atrophic gastritis and gastric adenocarcinoma induced by *Helicobacter pylori* infection. *Cancer* 1998;**82**:1013–18.
- Correa P. *Helicobacter pylori* and gastric carcinogenesis. *Am J Surg Pathol* 1995;**19**(suppl 1):S37–43.
- Correa P. Human gastric carcinogenesis: a multistep and multifactorial process—First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res* 1992;**52**:6735–40.
- Varis K, Taylor PR, Sipponen P, et al. Gastric cancer and premalignant lesions in atrophic gastritis: a controlled trial on the effect of supplementation with alpha-tocopherol and beta-carotene. The Helsinki Gastritis Study Group. *Scand J Gastroenterol* 1998;**33**:294–300.
- Go MF, Kapur V, Graham DY, et al. Population genetic analysis of *Helicobacter pylori* by multilocus enzyme electrophoresis: extensive allelic diversity and recombinational population structure. *J Bacteriol* 1996;**178**:3934–8.
- Achtman M, Azuma T, Berg DE, et al. Recombination and clonal groupings within *Helicobacter pylori* from different geographical regions. *Mol Microbiol* 1999;**32**:459–70.
- Covacci A, Censini S, Bugnoli M, et al. Molecular characterization of the 128-kDa immunodominant antigen of *Helicobacter pylori* associated with cytotoxicity and duodenal ulcer. *Proc Natl Acad Sci USA* 1993;**90**:5791–5.
- Parsonnet J, Friedman GD, Orentreich N, et al. Risk for gastric cancer in people with CagA positive or CagA negative *Helicobacter pylori* infection. *Gut* 1997;**40**:297–301.
- Atherton JC, Cao P, Peek RM Jr, et al. Mosaicism in vacuolating cytotoxin alleles of *Helicobacter pylori*. Association of specific vacA types with cytotoxin production and peptic ulceration. *J Biol Chem* 1995;**270**:17771–7.
- Boren T, Falk P, Roth KA, et al. Attachment of *Helicobacter pylori* to human gastric epithelium mediated by blood group antigens. *Science* 1993;**262**:1892–5.
- Boren T, Normark S, Falk P. *Helicobacter pylori*: molecular basis for host recognition and bacterial adherence. *Trends Microbiol* 1994;**2**:221–8.
- Ilver D, Arnqvist A, Ogren J, et al. *Helicobacter pylori* adhesin binding fucosylated histo-blood group antigens revealed by retagging. *Science* 1998;**279**:373–7.
- Gerhard M, Lehn N, Neumayer N, et al. Clinical relevance of the *Helicobacter pylori* gene for blood-group antigen-binding adhesin. *Proc Natl Acad Sci USA* 1999;**96**:12778–83.
- Sung JJ, Lin SR, Ching JY, et al. Atrophy and intestinal metaplasia one year after cure of *H. pylori* infection: a prospective, randomized study. *Gastroenterology* 2000;**119**:7–14.
- Siu LK, Leung WK, Cheng AF, et al. Evaluation of a selective transport medium for gastric biopsy specimens to be cultured for *Helicobacter pylori*. *J Clin Microbiol* 1998;**36**:3048–50.
- Miehlik S, Yu J, Schuppler M, et al. *Helicobacter pylori* vacA, iceA, and cagA status and pattern of gastritis in patients with malignant and benign gastroduodenal disease. *Am J Gastroenterol* 2001;**96**:1008–13.
- Yamaoka Y, Kodama T, Gutierrez O, et al. Relationship between *Helicobacter pylori* iceA, cagA, and vacA status and clinical outcome: studies in four different countries. *J Clin Microbiol* 1999;**37**:2274–9.
- Dixon MF, Genta RM, Yardley JH, et al. Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol* 1996;**20**:1161–81.
- Leung WK, To KF, Chan FK, et al. Interaction of *Helicobacter pylori* eradication and non-steroidal anti-inflammatory drugs on gastric epithelial apoptosis and proliferation: implications on ulcerogenesis. *Aliment Pharmacol Ther* 2000;**14**:879–85.
- Ifikhar SY, Steele RJ, Watson S, et al. Assessment of proliferation of squamous, Barrett's and gastric mucosa in patients with columnar lined Barrett's oesophagus. *Gut* 1992;**33**:733–7.
- Prinz C, Schoniger M, Rad R, et al. Key importance of the *Helicobacter pylori* adherence factor blood group antigen binding adhesin during chronic gastric inflammation. *Cancer Res* 2001;**61**:1903–9.
- Mizushima T, Sugiyama T, Komatsu Y, et al. Clinical relevance of the *babA2* genotype of *Helicobacter pylori* in Japanese clinical isolates. *J Clin Microbiol* 2001;**39**:2463–5.
- Odenbreit S, Puls J, Sedlmaier B, et al. Translocation of *Helicobacter pylori* CagA into gastric epithelial cells by type IV secretion. *Science* 2000;**287**:1497–500.
- Parsonnet J, Harris RA, Hack HM, et al. Modelling cost-effectiveness of *Helicobacter pylori* screening to prevent gastric cancer: a mandate for clinical trial. *Lancet* 1996;**348**:150–4.
- Moss SF. Cellular markers in the gastric precancerous process. *Aliment Pharmacol Ther* 1998;**12**(suppl 1):91–109.
- Yoshimura T, Shimoyama T, Tanaka M, et al. Gastric mucosal inflammation and epithelial cell turnover are associated with gastric cancer in patients with *Helicobacter pylori* infection. *J Clin Pathol* 2000;**53**:532–6.
- Moss SF, Calam J, Agarwal B, et al. Induction of gastric epithelial apoptosis by *Helicobacter pylori*. *Gut* 1996;**38**:498–501.
- Mannick EE, Bravo LE, Zarama G, et al. Inducible nitric oxide synthase, nitrotyrosine, and apoptosis in *Helicobacter pylori* gastritis: effect of antibiotics and antioxidants. *Cancer Res* 1996;**56**:3238–43.
- Jones NL, Shannon PT, Cutz E, et al. Increase in proliferation and apoptosis of gastric epithelial cells early in the natural history of *Helicobacter pylori* infection. *Am J Pathol* 1997;**151**:1695–703.
- Peek RM Jr, Moss SF, Tham KT, et al. *Helicobacter pylori* cagA⁺ strains and dissociation of gastric epithelial cell proliferation from apoptosis. *J Natl Cancer Inst* 1997;**89**:863–8.
- Fraser AG, Sim R, Sankey EA, et al. Effect of eradication of *Helicobacter pylori* on gastric epithelial cell proliferation. *Aliment Pharmacol Ther* 1994;**8**:167–73.
- Bechi P, Balzi M, Becciolini A, et al. *Helicobacter pylori* and cell proliferation of the gastric mucosa: possible implications for gastric carcinogenesis. *Am J Gastroenterol* 1996;**91**:271–6.
- Chow KW, Bank S, Ahn J, et al. *Helicobacter pylori* infection does not increase gastric antrum mucosal cell proliferation. *Am J Gastroenterol* 1995;**90**:64–6.
- Zychlinsky A, Prevost MC, Sansonetti PJ. Shigella flexneri induces apoptosis in infected macrophages. *Nature* 1992;**358**:167–9.
- Rokkas T, Ladas S, Liatsos C, et al. Relationship of *Helicobacter pylori* CagA status to gastric cell proliferation and apoptosis. *Dig Dis Sci* 1999;**44**:487–93.